Chemistry Data Sheet

EVOLUTE® CX Plates and Columns for Solid Phase Extraction in Bioanalysis

This Chemistry Data Sheet provides guidelines for the extraction of basic drugs from biological fluid samples using resin-based mixed-mode SPE. The generic method is described on page 1, with processing and method optimization guidelines on page 2.

An example application showing the extraction of basic drugs from plasma illustrates the versatility of EVOLUTE® CX when extracting a wide range of analytes (see Appendix).

EVOLUTE CX has been developed for the extraction of basic drugs from biological fluids. The resin-based mixed-mode sorbent is surface modified with well defined hydroxyl-functional oligomers, imparting excellent water-wettability. An optimized combination of non-polar (hydrophobic), polar (hydrophilic) and cation exchange interactions allows efficient extraction of basic drugs of wide ranging polarities. The non-polar / cation exchange dual retention mechanism allows the use of a rigorous interference regime, providing extremely clean extracts and reducing matrix effects associated with LC-MS/MS analysis.

Section 1: Methodology

This method is optimized for the 25 mg EVOLUTE CX plates. The method can be readily transferred to the 10 mg sorbent mass. See Section 5 Optimizing the SPE Method for details.

1. Sample Pre-treatment:	Dilute sample (typically plasma) 1:3 (v/v) with 0.05 M NH_4OAc, pH 6. Add internal standard (if used) and mix thoroughly.
2. Column Conditioning:	Condition each column or well with methanol (1 mL)
3. Column Equilibration:	Equilibrate each column or well with 0.05 M NH_4OAc, pH 6 (1 mL)
4. Sample Application:	Load sample (typically 400 µL* diluted plasma)
5. Interference Elution:	**Wash 1.** Elute interferences with 0.05 M NH_4OAc, pH 6 (1 mL) **Wash 2.** Elute interferences with methanol (1 mL)
6. Analyte Elution:	95:5 (v/v) methanol / NH_4OH (500 µL – 1 mL)
7. Post-extraction:	If desired, evaporate extract to dryness and reconstitute in mobile phase or other suitable solvent for analysis.

*Typical sample consists of 100 µL plasma diluted to a total volume of 400 µL. For larger volumes, see Section 5 Optimizing the SPE Method.

Biotage

Section 2: Reagents

0.05 M Ammonium Acetate Buffer, pH 6

Used in sample pre-treatment, equilibration and interference wash 1. Dissolve 3.854 g ammonium acetate in 950 mL deionized water, adjust to pH 6 using acetic acid (ACS reagent grade), mix thoroughly and make up to 1 L with deionized water.

95:5 (v/v) Methanol/Ammonia Solution

Used for analyte elution. Take 5 mL of 28% ammonium hydroxide and add 95 mL methanol. Mix thoroughly.

Section 3: Processing Conditions

Before starting the extraction procedure, set the vacuum levels to 1 – 2 "Hg for SPE columns, fixed-well plates, individual EVOLUTE Array wells, and both fully and partially populated EVOLUTE Array plates. This produces flow rates of 1 - 2 mL/minute for each format. For each step, load solvent or sample into all columns or wells prior to applying vacuum. This will ensure even flow rates and improved analytical precision.

Section 4: Maxiumum Sample Load

The volume of sample that can be extracted using EVOLUTE CX SPE columns and plates maybe restricted by liquid handling considerations. The maximum sample volume that can be applied to each format in a **single aliquot** is listed.

EVOLUTE CX fixed well plate:	2 mL (500 µL plasma at 1:3 v/v dilution)
EVOLUTE Array CX 1 mL wells and plates:	1 mL (250 µL plasma at 1:3 v/v dilution)
EVOLUTE CX 1 mL columns (inc. tab-less):	1 mL (250 µL plasma at 1:3 v/v dilution)

However, the capacity of EVOLUTE CX allows higher plasma volumes to be loaded without any loss of analyte. Exact volumes should be determined on a compound specific basis.

Section 5: Optimizing the SPE Method

a. For particularly viscous samples, increased sample dilution will normally improve flow characteristics.

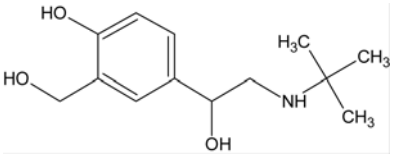
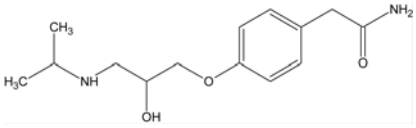
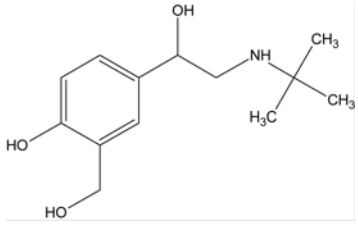
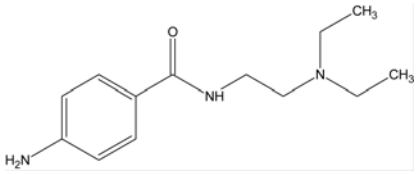
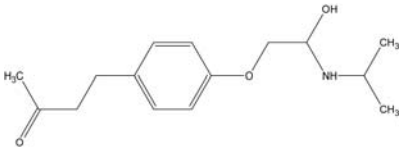
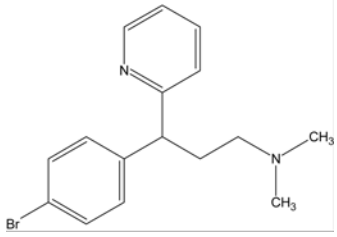
b. EVOLUTE CX is a water-wettable resin-based sorbent. Analyte recovery will be unaffected if the columns or wells run dry after conditioning.

c. Elution volumes can be minimized by the use of successive aliquots of elution solvent (e.g. 2 x 200 µL rather than 1 x 400 µL)

d. For 10 mg columns and plates, solvent volumes can be reduced to 500 µL for conditioning, equilibration and interference wash steps. The elution solvent volume can be reduced to 250 - 500 µL.

Using EVOLUTE CX with Alternative SPE Procedures

EVOLUTE CX is a versatile solid phase extraction sorbent and can be used with other manufacturers mixed-mode cation exchange polymer based SPE procedures, although further optimization may be required because of the subtle differences in retention and elution characteristics.

Analyte	Structure	Functionality	logP	pK_a
Salbutamol		Basic, polar	1.31	9.8
Atenolol		Basic, highly polar	0.16	9.1
Ranitidine		Basic, highly polar	0.27	8.8
Procainamide		Basic, highly polar	0.88	9.4
Metoprolol		Basic, polar	1.88	10.8
Brompheniramine		Basic, non-polar	4.06	3.59 and 9.2

[1] pK and logP values were obtained from literature or values were calculated if not available

Basic drugs spiked in plasma (100 µL) at concentrations of 50 ng/mL, using EVOLUTE CX 25 mg plate and SPE method as described in **Part 1**. Analysis was by LC-MS/MS. Request Application Note **AN702** Extraction of Basic Drugs from Plasma using EVOLUTE CX SPE Columns for full sample preparation and analytical methodologies.

APPENDIX 1

Extraction of Basic Drugs from Plasma

Using a single generic method, EVOLUTE CX is suitable for extraction of basic drugs with wide ranging polarity. **Figure 1** shows the results of the extraction of a basic analyte suite exhibiting a wide range of polarities. See **Table 1** for analyte structures, logP and pK_a data.

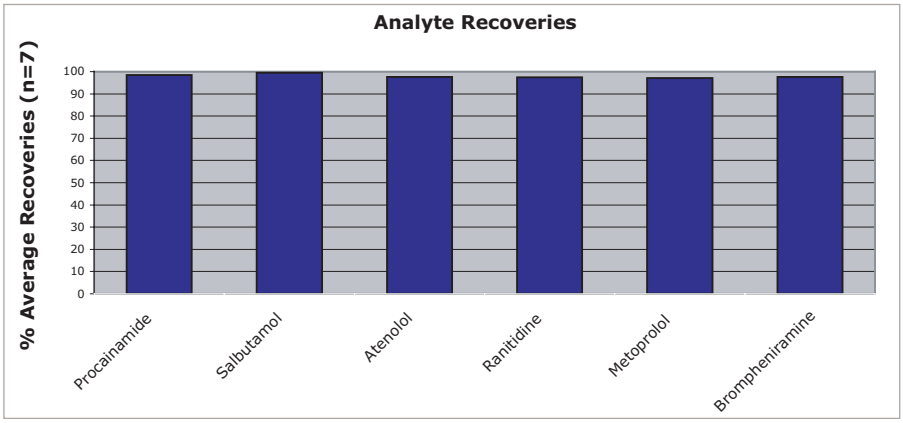


Figure 1. EVOLUTE CX provides high absolute recoveries (>85%) with excellent reproducibility (<10 % RSD, n=7) for a selection of basic drugs from plasma at a concentration of 50 ng/mL

Reproducible Extraction Performance

Fines free sorbents are an important feature of a high quality SPE product. The particle size distribution of EVOLUTE CX is carefully controlled during manufacturing and quality controlled to ensure a narrow distribution optimal for bioanalytical SPE. This minimizes fines and maximizes the packing efficiency and subsequent performance of the SPE column. **Figure 2** shows the particle size distribution of EVOLUTE CX.

Stringent QC tests are carried out during manufacturing, ensuring the extracted sample is not contaminated with sorbent fines or impurities from the SPE column components.

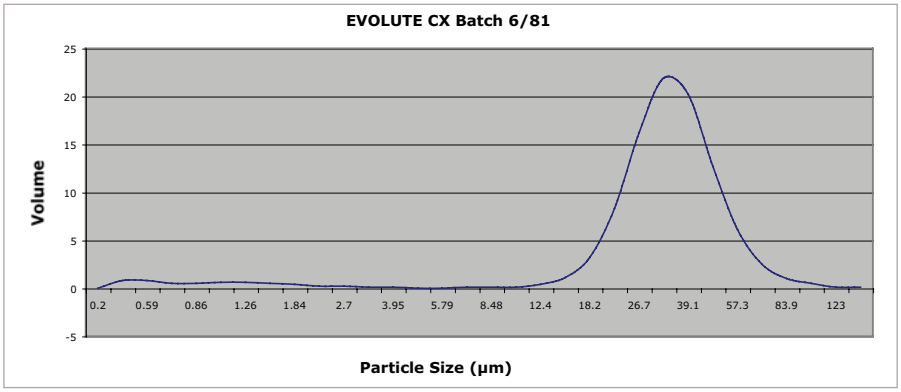


Figure 2. Particle size distribution of a batch of EVOLUTE CX

Demanding QC testing, including the use of LC-MS/MS analysis for a carefully selected suite of basic drugs ensures consistent performance from batch-to-batch. **Figure 3** shows the reproducibility across three batches of EVOLUTE CX.

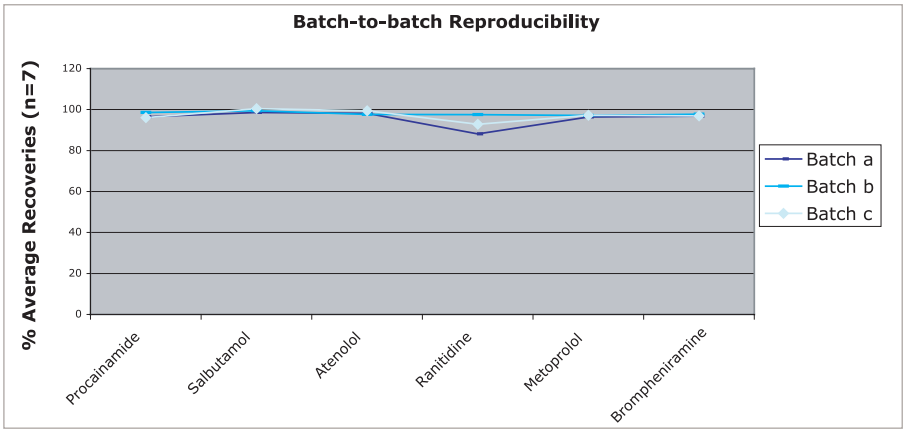


Figure 3. Extraction of basic drugs at concentration of 50 ng/mL from plasma from EVOLUTE CX 25 mg plates manufactured using three different batches of sorbent.

Processing Options
EVOLUTE CX SPE Plates and Columns are compatible with manual and automated sample processing. Contact Biotage for details on the range of VacMaster™ Sample Processing Manifolds for manual processing.

APPENDIX 2

ORDERING INFORMATION

Description	Quantity	Part number
EVOLUTE CX Fixed Well Plate		
EVOLUTE CX 10 mg Fixed Well Plate	1	601-0010-P01
EVOLUTE CX 25 mg Fixed Well Plate	1	601-0025-P01
EVOLUTE CX Array Plates and Wells		
EVOLUTE Array CX 10 mg/1 mL wells	100	601-0010-R
EVOLUTE Array CX 25 mg/1 mL wells	100	601-0025-R
Pre-assembled EVOLUTE Array plates are available. To order, add the suffix P to the equivalent loose well part number. e.g. 601-0010-RP		
EVOLUTE Array Accessories		
EVOLUTE Array base plate	1	120-6000-P01
Strip of 8 base plate sealing plugs*	50	120-1200
Luer adaptors (fits standard sample processing manifold)	25	120-1201
Well removing tool*	1	120-1202
*Required when processing a partially populated EVOLUTE Array CX plate.		
EVOLUTE CX 1 mL SPE Columns		
EVOLUTE CX 10 mg/1 mL SPE Columns	100	601-0001-A
EVOLUTE CX 25 mg/1 mL SPE Columns	100	601-0002-A
EVOLUTE CX 1 mL SPE (Tab-less) Columns		
EVOLUTE CX 10 mg/1 mL Tab-less SPE Columns	100	601-0001-AG
EVOLUTE CX 25 mg/1 mL Tab-less SPE Columns	100	601-0002-AG

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